



# Chronic Intermittent Ethanol (CIE) Exposure Model

Mouse Model of Ethanol Dependence and Relapse Drinking  
Chronic Intermittent Ethanol (CIE) Exposure Model  
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## Subjects

This model has been predominantly been studied using adult (65-75 days of age) male C57BL/6J mice obtained from Jackson Laboratories (Bar Harbor, ME). Although experimental parameters have been optimized using these mice, we have conducted studies with adult female C57BL/6J mice, as well as other inbred mouse strains including DBA/2J and C3H/HeCr, selectively bred HAP/LAP lines, and and BXD RI lines. Mice are acclimated to the facility for at least two weeks prior to experimental use. Mice are individually housed in standard polypropylene pans with wood-chip bedding and stainless steel wire lids. The mice are housed under a modified light/dark cycle (lights on at 0200 hr) to allow for behavioral testing to be conducted during the dark phase of the cycle. Rodent food (Wayne Lab-Blox) and water is available ad libitum at all times during the studies.

## General Experimental Design and Strategy

The overall objective of this chronic intermittent ethanol (CIE) exposure model linked to a limited access drinking procedure is to enable comparison of voluntary drinking in dependent (CIE-exposed) mice with consumption in nondependent mice. The general experimental strategy of this model is to first establish the positive reinforcing effects of ethanol and then evaluate the influence of CIE exposure in facilitating the negative reinforcing effects of the drug. All mice are trained to voluntarily drink ethanol in a 2-bottle choice situation (15% v/v ethanol vs. water) using a home cage limited access (2 hr/day) paradigm.

Once stable baseline ethanol intake (g/kg) is established ( $\leq 15\%$  variance in daily intake over a week), mice are separated into two groups (equated for baseline alcohol intake). One group (EtOH group) is exposed to chronic intermittent ethanol vapor (16 hr/day x 4 days) in inhalation chambers, as described below. The remaining mice (CTL group) are similarly treated, but exposed to air in control inhalation chambers. Home cage limited access to ethanol is suspended while the mice receive their respective treatments in inhalation chambers. Starting at 72 hr following the final chronic 16-hr ethanol vapor (or air) exposure bout (i.e., a time corresponding to when physical signs of acute withdrawal have resolved), ethanol limited access drinking sessions resume for five consecutive days. The four 16 hr/day exposure bouts followed by a 72 hr forced abstinence period is considered a single exposure cycle. Thus, each weekly exposure cycle is followed by a 5-day limited access drinking test cycle. Typically, this procedure is repeated for four cycles (yielding 4 exposure cycles and Test Cycles 1-4).

## Limited Access Drinking Model

Using a modified sucrose-fading technique (Samson, 1986), mice are given daily access to ethanol for 2 hr in the home cage, beginning at 0.5 hr prior to the start of the dark cycle (lights off at 1400 hr). Standard water

bottles are removed and replaced with 15- ml graduated tubes containing an appropriate ethanol/sucrose solution and a bottle containing tap water as an alternative fluid. Mice receive, in sequence, for two days each: 10% ethanol/5% sucrose, 12% ethanol/5% sucrose, 15% ethanol/5% sucrose, 15% ethanol/2%, and 15% ethanol/1% sucrose. The mice are then maintained on 15% ethanol (v/v) with no sucrose for the remainder of the study. The daily limited access drinking sessions occur 5 days/week (Monday through Friday) throughout the entire study. At the end of the daily 2 hr access periods, ethanol/sucrose and water bottles are removed and standard water bottles are returned to the home cages. The position of the ethanol and water tubes is alternated on a daily basis to avoid side preference. Red lights in the colony room minimize disturbance of animals during their dark cycle. Body weight is recorded weekly and ethanol and water intake is measured daily (to the nearest 0.1 ml). Solutions are presented at room temperature and prepared fresh each day by mixing ethanol (95% ethanol) and sucrose with deionized water to arrive at appropriate ethanol (v/v) and sucrose (w/v) concentrations. Throughout all phases of the study, mice are neither food nor water deprived.

The model typically involves 2 weeks of sucrose fading followed by 4 weeks of establishing baseline intake. The long baseline period is designed to establish stable intake prior to entering the CIE exposure phase of the study. The last week of baseline is typically used in analyses to compare with intake during Test Cycles. Baseline intake for male C57BL/6J mice is usually in the range of 2.5-2.75 g/kg. This range typically will enable observing a significant escalation of intake in CIE-exposed mice. Baseline intake below 2.0 g/kg may indicate general aversion to ethanol and intake above 3.0 g/kg may make it difficult to observe significant increases in the CIE-exposed group.

Dependent variables to be analyzed include ethanol intake (ml and g/kg), water intake (ml), and total fluid intake (ethanol + water intake). Data is commonly expressed as weekly average intake (determined for each subject) and analyzed by ANOVA with Group (CIE vs CTL) as a between-subjects variable and Test Cycle (including Baseline) as a repeated measure. Analysis of daily intake is similarly analyzed with the inclusion of Day as a repeated measure as well. The ethanol concentration (15% v/v) is chosen because it represents a relatively high concentration that C57BL/6J mice will readily consume in a relatively short period of time. The session duration (2 hr) is chosen because total amount of ethanol consumed (g/kg) within this time frame is tightly correlated with blood ethanol levels registered immediately after the limited access period.

### Chronic Intermittent Ethanol (CIE) Exposure Procedure

Induction of ethanol dependence is accomplished through chronic intermittent administration of ethanol by the inhalation route. The design of the inhalation chambers and procedures for chronic alcohol exposure are similar to that described in our published work (Becker and Lopez, 2004; Griffin et al., 2009a; Lopez and Becker, 2005). Briefly, mice are placed in custom-built Plexiglas inhalation chambers (60 x 36 x 60 cm), with the housing conditions similar to that in the colony room. Ethanol (95%) is volatilized by passing air through an air stone (gas diffuser) submerged in the ethanol. The ethanol vapor is mixed with fresh air and delivered to the chambers at a rate of 5 L/min. This maintains the ethanol concentration in the chamber in the range of 18-22 mg/L air and yields blood ethanol levels in the range of 175-225 mg/dl during exposure. This degree of intoxication ( $\geq 175$  mg/dl BEC) maintained during each exposure appears critical for favoring significant escalation of drinking in CIE-exposed mice (Griffin et al., 2009b).

CIE exposure is accomplished by exposing mice to ethanol vapor for four days (16 hr/day), with 8 hr periods of withdrawal separating each exposure bout. The first day of exposure starts on Monday afternoon. This pattern of 16 hr intoxication followed by an 8 hr period of abstinence is repeated for four bouts. The 8 hr withdrawal period is sufficient in length to allow ethanol to be cleared before the next cycle of intoxication begins (to avoid ethanol exposure from "bleeding" into the next bout of exposure) (Becker, 1994). A separate group of mice is similarly handled but maintained in control (air) chambers throughout all cycles of the study. At the beginning of each exposure bout, ethanol intoxication is initiated by intraperitoneal (IP) administration of 1.6 g/kg ethanol (8% w/v), and blood ethanol concentration is stabilized by injection of the alcohol dehydrogenase inhibitor pyrazole (1 mmol/kg; IP). Mice maintained in control (air) inhalation chambers receive an injection pyrazole along with saline rather than ethanol. In this way, all EtOH and CTL groups are handled similarly and receive the same number of injections.

### Ethanol Samples and Measurement Procedures

Chamber ethanol concentrations are monitored daily and air flow is adjusted to maintain ethanol concentrations within the specified range. Ethanol concentrations in inhalation chambers are determined using a portable breath analyzer system (Lifeloc, Model FC10). Air samples (5 ml) are collected with a 60-ml syringe (fitted with a 1.5 in, 23 gauge luer-lock needle) inserted through a port in the chamber wall. The air samples are diluted (1:12) with fresh (room) air and then injected into the modified mouthpiece of the breath analyzer (fitted with a rubber stopper) at a steady rate (uniform pressure). Breath analyzer values (x) are recorded and then later converted to ethanol chamber concentration (mg/L) using the following linear equation: [EtOH] mg/L = 62.52x + 6.18. This is based on regression analysis of ~60 samples (R<sup>2</sup> = 0.79; p < 0.0001) correlating inhalation chamber ethanol concentrations determined by breath analyzer vs. an enzymatic spectrophotometric assay procedure based on the Calbiochem-Behring method (La Jolla, CA), as previously described (Becker and Hale, 1993). This alternative method also involves collecting air samples from the chamber, but in this case samples are transferred to Venoject<sup>TM</sup> tubes and mixed with reagents for this enzymatic spectrophotometric assay that relies on the stoichiometric reduction of NAD to NADH<sup>+</sup> as a by-product of ethanol oxidation via alcohol dehydrogenase (Becker and Hale, 1993).

Additionally, to avoid stress of blood sampling influencing behavioral (drinking) and other endpoint measurements, blood ethanol concentrations (BEC) following inhalation exposure is typically determined in a separate group of similarly treated sentinel mice (N = 3-4 mice/chamber). At least once during each cycle of chronic intermittent exposure, blood samples are collected from the sentinel mice for subsequent BEC analysis (targeted BEC = 200 ± 25 mg/dl). Blood samples are collected from the retro-orbital sinus using heparinized capillary tubes. The whole blood samples (~20 µl) are collected on ice, transferred to microcentrifuge tubes and centrifuged at 14,000 x g for 10 min for phase separation. Aliquots of plasma (5 µl) are then injected into an Analox Instruments analyzer (Lunenburg, MA) for determination of BEC (detected by measuring oxygen uptake generated by the oxidation of ethanol into acetaldehyde and hydrogen peroxide by alcohol oxidase). BEC values are expressed as mg/dL blood.

## References

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